SEX RECOGNITION IN THE GUPPY, LEBISTES RETICULATUS PETERS

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(Figs. 24 and 25)

INTRODUCTION

Descriptive studies of the reproductive habits of fishes comprise a large literature but analytical consideration of the factors involved is relatively scant. This paucity of critical examination is especially marked in the matter of sex recognition. Those papers which do go into the subject at all are confined to a discussion of species in which nest building or some other intricate behavior pattern is an accompaniment of mating. In such cases the females are necessarily obliged to take some active part in the reproductive act, since they are oviparous and fertilization is coincident with the shedding of the female genital products. The species at present under consideration, Lebistes reticulatus Peters, differs from these in that it represents a group of viviparous fishes in which mating takes place at a time prior to extrusion of the genital products from the female and in which the act of fertilization is successful without any apparent cooperation on the part of the female.

Considering the findings on oviparous fishes, the actual act of recognition is based chiefly if not entirely on the difference in behavior between a female ready to spawn, and others. This appears again and again in various guises, depending on the physical equipment of the specific form and its particular mating requirements. Such studies on the lamprey have been made by Young and Cole (1900) and Reighard (1903); on a darter by Reeves (1907); on a dace by Smith (1908); on the chubs and minnows by Reighard (1910 and 1920); on the log perch (1913) and on the stickleback by Wunder (1927 and 1930); on the

Siamese fighting fish by Lissmann (1932); on a cichlid by Breder (1934) and on a sunfish by Noble (1934). In addition, various unpublished observations have been made on Cyprinidae, Siluridae, Labyrinthidae and Centrarchidae which indicate the same type of behavior. It is unnecessary to enter upon a full discussion of sex recognition in such forms at present, and this mention is made chiefly to call attention to the differences in the mode of reproduction in such forms and the one under consideration. The fish *Lebistes* presents a most striking form of sexual dimorphism concerning color, pattern, body form and size. It is, in fact, much more marked than in any of the abovementioned species that have been published on or studied. Nevertheless, cooperation by the female is not an apparent element.

The reason for undertaking the present study was to determine if the methods of recognizing a suitable mate in *Lebistes* differed in accordance with the physical differences from other species and with other matters concerning reproduction. These include the mode of mating, the role of sexual dimorphism, the significance of the courtship and the attitude of the female toward courting males.

The mating of Lebistes may be described as follows: An active male on approaching a female usually spreads his fins widely, bends his body slightly and vibrates, accompanying this by a curious backing motion. This usually takes place slightly below and to one side of the head of the female but may occur in almost any spot relative to the female. Almost always this is interrupted by the female swimming rapidly away. In a small aquarium, with few fish, a more or less vigorous pursuit may follow. More often, however, the male is distracted by other females which he then proceeds to court. Under usual aquarium circumstances the males are generally outnumbered about two to one, as shown by Breder and Coates (1932). The behavior described above is commonly accompanied by a more or less energetic movement of the exceedingly mobile gonopodium to the side next to the female. This behavior may almost always be found in a tank of Lebistes. Normal, healthy males seem to be almost continually active in this regard, interrupting it only for feeding, but without considerable observation this is about all that can be usually noted in such an aquarium. Prolonged observation will reveal, however, that eventually the male gives up this procedure and directs a rather violent thrust of the gonopodium toward the genital pore of the female. A momentary contact effects the transfer of the encapsuled spermatozoa. This actual transfer of material seems only to occur after the male has slipped up to the seemingly unsuspecting female. Not infrequently a male may be seen to court one fish and as she flees succeed in fertilizing another and hitherto unnoticed one. No females at any time have been observed to show other than escape reactions to the male attentions. Never were they observed to evince the slightest evidence of interest in the proceedings. The significance of these elements of the reproductive act are examined in the discussion.

The experimental parts of this study were directed toward the actual modus operandi of sex recognition on the part of the male Lebistes.

EXPERIMENTAL STUDIES

Preliminary to the experiments, males were isolated in aquaria from which they could not see other fishes for a period of at least six days, and fed adequately with Daphnia. was done on the supposition that such confinement would insure an active "sex appetite," although it must be admitted that male guppies have never been noted by the authors to be deficient in that regard. The males were then placed in a series of observation chambers, one fish to each. There were six "stalls," each large enough to hold one rectangular battery jar (5"x3"x8") completely shielded from outside interference, lighted from the top, and with a carefully screened observation peep-hole cut through one wall. To these fishes various stimuli, such as females, were introduced in different manners. For purposes of analysis the reaction of the male was considered positive when the male erected the gonopodium, vibrated the dorsal fin and displayed directly before or in the immediate vicinity of the stimulus, whether or not copulation was effected. All other activity was considered negative. The responses were noted and timed. In every case the stimulus was removed from the subject after four minutes had elapsed, except as otherwise noted. No response in that period was entered as negative. Not more than four tests of a subject were made in any one day and a period of at least thirty minutes was allowed between each experiment on any one fish. As will be further developed, this was necessary because of a peculiarly rapid conditioning that early became apparent.

Reactions to female Lebistes: As is well known, male Lebistes normally court females on sight. Consequently, it is not surprising that in all cases positive reactions were secured when females were presented directly (Test 1, Table I). Females floated in a small beaker gave less than 100% response. Of the 78 tests, 48, or 62%, elicited a positive reaction (Test 2, Table I). Females exhibited in another aguarium placed beside the test aquarium resulted in only a 17% response (Test 3, Table I). When removed to a distance of 150 mm., no response was obtained (Test 4, Table I). This series of tests shows clearly that vision alone may serve to account for sex recognition in Lebistes. As a check on this, females were placed in the aquarium confined in a perforated but opaque container (Test 9, Table I), and water from an aquarium containing females was added (Test 10, Table I). Both yielded no response, indicating the lack of a possible chemical stimulation operating in sex recognition. See also Table IV, which gives the data of Table I recalculated in detail showing the behavior of individual males to their various trials.

It is to be noted that the percentage of response falls from 100% to 0% in tests Nos. 1 to 4. If the average random positions possible for the female in relation to the test male are plotted, a chart expressing this relationship may be constructed. Such a diagram is given in Figure 24. Thus Test 1 is practically 0 distance because of the small size of the test aquarium. Test 2 had an average distance of 50 mm. This is further complicated by the partial obscuration of vision due to the curving of the beaker as well as its position more or less above the test male, because of its being floated in the aquarium. Test 3 had an average distance of 100 mm. (center to center of the two tanks). Test 4 similarly represents a measured distance (center to center). The line "Female Lebistes," in Figure 24, probably thus represents merely a falling off of visual acuity with distance.

This may be further demonstrated with any aquarium of *Lebistes*. Practically any object moved in front of such an aquarium will attract all the fishes to that side if the object is not more than 150 mm. away. This, obviously, refers to conditions of bright light falling in such a direction as not to cast a shadow on the aquarium. In the latter case distance of object has little to do with visibility. In conditions of poor light or slight difference between color of object and background, the distance of visibility is less.

Females anesthetized with chloretone, lying on the bottom of the aquarium (Test 5, Table I), and suspended by a hair so that some imitative motion was possible (Test 6, Table I), both produced some response. It is perhaps remarkable that the first gave a 53% response, while the second, with motion, gave only 14%. It may be that the movements were so unlifelike that some fright was induced (?). It is to be noted that the presumable exudation of the chloretone did not inhibit attempts at mating, again emphasizing the lack of a chemical element in matters of sex recognition.

Freshly dead, suffocated females, direct in the test aquarium (Test 7, Table I) or in the beaker (Test 8, Table I), failed to evoke the mating reaction. In the former, three out of twelve test males attempted to feed on the dead female. At this writing it is not clear just how this "food recognition" operates, or how the difference between an anesthetized and a dead fish is detected.

Reactions to other fishes: Since Lebistes have been seen to attempt to mate with other males, especially if the latter were large, and with other fishes, no tests were made with males directly in the same aquarium. Males were exhibited in the beaker (Test 11, Table I) and produced a large percentage of positive reactions, 75%, while females under the same conditions produced only 62% on the same test males. As male Lebistes are rather more active than the females, it may be that under such conditions the former are simply more conspicuous.

Three foreign species—Cyprinodon, Barbus and Fundulus—tested direct and in the beaker gave rather interesting results.

¹ Chloretone 1 cc. sat. sol. to 5 H₂O. The reaction period averaged about two minutes and recovery occurred in about thirty minutes. No mortality or ill effects were noted.

TABLE I Results of Exposures of female Lebistes under various conditions to 24 test males in 179 trials

Exp. No.	Exposure to test male of:	No. of Tests	No. Pos.	No. Neg.	% of Tests Positive
1	Female Lebistes direct in same aquarium	36	36	0	100
2	Female Lebistes in a beaker floated in aquarium.	78	48	30	62-
3	Female Lebistes in an immediately adjacent aqua-				
	rium	6-	1	5	17—
4	Female Lebistes in an aquarium 15 cm. distant	6	0	6	0
5	Anesthetized female direct in same aquarium,				
	lying on bottom	15	8	7	53 +
6	Anesthetized female direct in same aquarium,				
	suspended by hair	7	1	6	14 +
7	Freshly dead female direct in same aquarium		0	12	0
8	Freshly dead female in a beaker floated in aqua-				
	rium	6	0	6	0
9	Female in perforated opaque box in aquarium	7	0	7	0
10	Water from aquarium containing many females				
	added to aquarium	6	0	6	0

Results of Exposures of male Lebistes and fish of other species, under various conditions to 12 test males in 72 trials

11	Male Lebistes in a beaker floated in aquarium	12	9	3	75
12	Cyprinodon variegatus direct in same aquarium.	6	3	3	50
13		12	3	9	25
14 15	Fundulus heteroclitus direct in same aquarium Cyprinodon variegatus in a beaker floated in	12	4	8	33+
	aquarium	6	1	5	17—
	rium	12	8	4	67-
17	Fundulus heteroclitus in a beaker floated in aquarium	12	7	5	58-

Results of exposure of models, shadows and other objects under various conditions to 12 test males in 78 trials

18	Model of female Lebistes suspended immediately				
	outside aquarium	6	0	6	0
19	As in 18, but moving	6	0	6	0
20	As in 18, but suspended in side aquarium	6	0	6	0
21	As in 20, but smeared with mucus from living				
	female	12	0	12	0
22	As in 21, but moving	6	0	6	0
23	Mirror attached to outside of aquarium	12	0	12	0
24	Empty beaker floated in aquarium	18	2	16	11+
25	Projected shadow of living fish on screen at-				
	tached to aquarium	12	2	10	17—

All gave a percentage of positive reactions. The degree of activity of these three fishes is in the ascending order of Cyprinodon, Fundulus, Barbus. The percentage of response direct in the aquarium was in the reverse order of this: 50%, 33%, 25% (Tests 12, 13, 14, Table I). The active and fast moving Barbus scarcely gave the male a chance to organize its courting display before it was off in another corner with the male in pursuit. The more sedate Cyprinodon usually permitted the male to go through a recognizable positive display before moving off. Fundulus was somewhat between these two. When confined in the beaker an inversion of these relationships was found; i.e., the order of reaction stood Barbus, Fundulus, Cyprinodon, with reactions 66%, 58%, 16%, respectively (Tests 16, 17, 15, Table I). These fish closely confined in a beaker had scant range of movement but preserved their specific degree of activity. This, then, instead of acting as deterrent as before, attracted greater attention in a manner analogous to that in which a male Lebistes in a beaker attracted more than a female (Tests 2 and 11, Table I). From this it may be fairly inferred that degree of activity and movement are important in stimulating mating activity.

Reactions to other objects: A very carefully made model of a female Lebistes² was tested in various ways but in no case was a response obtained. It was suspended quietly outside the acuarium (Test 18, Table I) and with movement (Test 19, Table I). It was suspended in the aquarium without motion (Test 20, Table I) and was smeared with mucus of a living female, still (Test 21, Table I) and moving (Test 22, Table I). These latter two experiments again indicate the lack of involvement of a chemical sense. Like the distinction of a dead from an anesthetized female, the lack of courting of this model is not explainable at this writing. Certainly other fishes will attempt displays before models. For example, Betta splendens Lissmann (1932) and Eupomotis gibbosus Noble (1934).

A mirror placed outside of the aquarium produced no response, but mirrors in an aquarium will do so frequently (not part of Table I). Lissmann has also noted this for *Betta*. This is referred to the apparent distance of the mirror image rather

² We are indebted to Mr. Edward Howell, sculptor of miniatures, for the preparation of this model.

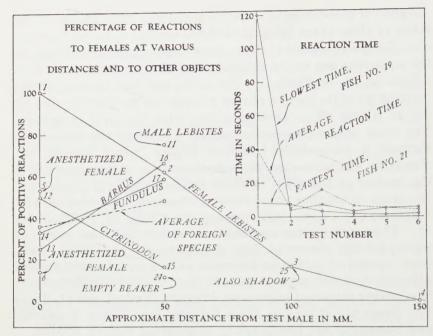


Fig. 24. Graphic arrangement of reactions of male Lebistes in a study of sex recognition.

Main graph: Percentage of courting reactions of test males to objects named in italics. Numbers in italics refer to the test numbers of Table I. Test yielding zero reactions omitted, except to female Lebistes.

Insert graph: Time in seconds for test males to display courtship activities in successive trials. Italics indicating fastest, slowest and average time refer to Table II.

than any other factor, as suggested by the data on the female reactions shown in Figure 24. For example, the mirror's actual distance was about 100 mm. but the apparent distance was about twice that.

A projector was so arranged as to allow a narrow aquarium to be placed between the lens and the light source. The silhouette image of a fish placed in this tank was projected on a piece of parchment affixed to the side of the test aquarium. For this purpose a *Barbus* was used, because of its activity. The response elicited by this image was exactly the same as brought forth by a female at the same distance, 17% (Test 25, Table I). See Figure 24.

A most peculiar response was obtained in checking the possible extraneous effects of introducing a beaker into the test

aquarium. This was introduced eighteen times empty. On two such trials a positive reaction was obtained (Test 24, Table I). Two males out of the six so tested reacted. This reaction included thrusting the gonopodium vaguely at the curve of the bottom and side of the beaker, at the place where the females usually come to rest. This is referred to a rapid conditioning, since it only occurred after the males in question had been attracted by a beaker containing a female.

The details of this behavior are set forth in Table II. These data arranged graphically are given in Figure 25. It will be noted that the closely analyzed data, considering the reactions to females in beakers alone, rose from 0% to 100% in three test periods at similar times on three successive days. likely the failure to respond at first had to do with initial fright on the disturbance of introducing the beaker. As this passed off, the attractive powers of the contained female very rapidly overcame it, coupled with a conditioning to a repeated stimulus that was followed by no "punishment." Four days later females were again presented in this fashion and then only two of the six males, or 33+%, reacted.3 Apparently in that time the conditioning had partly disappeared, or, at least, the fish had forgotten the association of a possible mate with this type of disturbance. Males presented at 4 P.M. of the last day (8/16) caused a response by five of the six males, or 83+%. This is not thought to be a significant difference, since on other tests (see Table I and Figure 24) the males, considering all tests, showed a stronger attraction, as is discussed in another place. Fish Nos. 7-12 inclusive were exposed to a male in a beaker, a male in a beaker outside the aquarium, and twice to females in beakers in the aquarium in successive hours, and to females in a beaker twenty-four hours later. Comparable results were obtained, considering the slightly differing conditions:

The first exposed (male) and the third and fourth (female) induced a comparable increase in percentage of the test males reacting; i.e., 66+%, 83+% and 100%. The second (male) is not comparable, for the fish was farther away and the percentage was proportionately lower, 16+%. Compare with data

³ These data and that following on this subject were not included in tabular matter because of space limitations.

TABLE II

Conditioning of males to a female in a beaker floated in an aquarium and to an empty beaker. Fishes No. 1 to 6, inclusive, used once in each test

Beaker	% of Tests Positive	No. Negative	No. Positive		Date and of test ()
with femal	0	6	0	2	8/14
with femal	17—	5	1	3	8/14
with femal	33+	4	2	4	8/14
with femal	33+	4	2	5	8/14
empty	17—	5	1	3	8/15
empty	0	6	0	4	8/15
with femal	83+	1	5	5	8/15
empty	17—	5	1	1	8/16
with femal	100	0	6	2	8/16
with femal	100	0	6	3	8/16

in Figure 24. The higher level of the first three as compared with data in Figure 25, would seem to be referable to initially less fear on the part of these fish or earlier unintentional conditioning of which no accurate record was kept. Twenty-four hours later a female in a beaker elicited a 66+% response. This set seemed to unlearn what they had learned the day before, whereas the first set of test fish did not. Such differences are naturally to be expected and, if anything, these figures are rather remarkable for their closeness of agreement.

Referring again to Table II and Figure 25, the remarkable response to an empty beaker may be examined. Presenting such a beaker about twenty-four hours after a response to a female, a 16+% reaction was obtained. An hour later it was zero. An hour following this the recognition of a fish in the beaker was demonstrated by 83+%. The next day again, about twenty-four hours later, 16+% was again obtained. An hour later 100% reaction was the response to females in the beaker. From this it may be inferred that the association of a female with a beaker is retained for at least twenty-four hours, but one presentation of the empty beaker is sufficient to break this. It may be built up again on one exposure to a female in the beaker. This discussion could be carried somewhat further, considering the length of time before a positive reaction took place, etc., but it

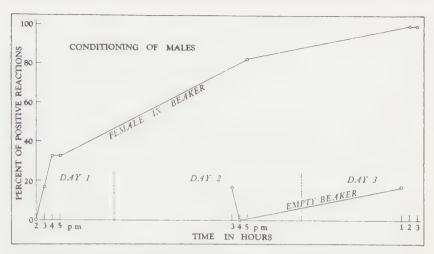


Fig. 25. Graphic arrangement of conditioning of male Lebistes to females in a beaker floated in their aquaria, and to empty beakers. The data refer to Table II. Each point represents the percentage of positive responses obtained from one test on each of six fishes (Males No. 1 to 6, inclusive).

may suffice to point out that the reactions to the empty beaker were rapid as compared with an average of the others. Further experiments would be necessary to demonstrate more thoroughly the extent of this apparent "snap judgment" and conditioning, but for the present purposes the above will suffice. It demonstrated the need of care in a study of this kind, which was its only purpose. All subsequent work was carried on with these data as a guide, involving the application of time intervals sufficient to assure the unlearning of any possible conditioning.

This phenomenon leads to an examination of the speed of the reaction times of the males of this species. Six males were exposed to a female direct in their aquaria, six times each. The time in seconds for each reaction is given in Table III. Between each test a period of twenty-four hours elapsed, except between Tests 4 and 5, which was forty-eight hours. It will be noted that the mean reaction time varied from twenty-four seconds (Fish No. 19) to four seconds (Fish No. 21). The average of the reaction times for each successive trial falls in good order from forty seconds to four seconds. Fish No. 20 did not react rapidly on the third trial and then seemed to begin all over. Omitting this one exceptional fish, the curve of descent

would be even more regular. The inset of Figure 24 gives the average reaction time, together with that of the fish with the longest and shortest mean time. It is to be noted that after the initial drop between Tests 1 and 2, there is little further reduction. The introduction of a female from a net seems to be taken for granted almost after one trial. Compared with the "learning curves" of Welty (1934) for goldfish, the present would seem to be in accord, considering the large difference between maze learning and sex recognition which make use of the food and mating "drive," respectively. This is in keeping with the

TABLE III

Reaction Times of Experiment No. 1, Table I

Test Male No.	1	Trial No.	Reaction 3	Time in	Seconds 5	6	Average
19	120	4	7	3	5	5	24
20	5	7	75	25	11	7	22—
21	8	7	3	2	1	2	4
22	72	2	1	2	2	3	14—
$\overline{23}$	11	4	7	1	2	5	5
24	14	$\bar{2}$	4	3	7	2	5
VERAGI	$\equiv \frac{-}{40}$	4+	$\frac{-}{16+}$	6	- 5—	4	

speed of learning that *Lebistes* show, regarding in which corner of the aquarium they are commonly fed. We consider this an explanation of the attempt to court an empty beaker by some rapidly learning males, especially since there appears to be considerable spread in the rapidity with which *Lebistes* learn, as is evidenced by data given in Tables II and III.

Discussion

It is clearly evident from the foregoing experiments that vision alone can account for the marked sexual activity of *Lebistes*. Experiments involving the chemical senses, on the other hand, yield nothing but negative results. The same is true of any conceivable mechanical agitation. Experiment No. 9 should have given some such evidence on this sense, as well as olfaction if it were present. In this connection it is noteworthy

that *Lebistes* are purely diurnal and attempt no mating or courting at night, as may be noted by suddenly flashing on a light or examination by a dull red light to which they are not responsive. When the light falls below a certain threshold, they quiet down and rest passively, usually in the shelter of some vegetation.

The items calling forth the display reactions of the courting male may be evoked by a wide variety of optical stimuli so long as they occur within a limit of about 150 mm., provided the light is not behind the subject. This latter, naturally, is rare in a state of nature and probably does not enter at all. It could occur only with an object overhead and since *Lebistes* is so predominantly a surface fish of shallow water, such an occasion would certainly be uncommon.

The apparent distinction between a dead female, a model and an anesthetized one, is not readily explained. Parts can be explained on a reasonable basis, however. Since the males will attempt courting a projected shadow, it may be that the "characteristic" fish movement with its apparent alternate expansion and contraction in size is the important factor. Then, all the experiments involving a moving model, stiff and awkward, might be ruled out as fear-inspiring, rather than attractive. This in no way, however, helps in understanding why a dead female lying on the tank floor was treated as a food object, while a similarly inert anesthetized one was courted. While this peculiarity requires further study, it certainly suggests the entry of some delicate chemical distinctions. Under water, the otherwise disregarded "effluvia" of a dead fish is very likely different from that of one under an anesthetic, although one would suppose that such a substance itself would act as a repellent.

Considering the visual elements involved, we seem to be on much more secure ground. These reactions of the male are clearly conditioned purely by the size, distance and amount of motion of the object involved, as modified by the light conditions affecting the visual acuity of the subject. It is only at exceedingly close ranges that certain features of recognition become confused, as above indicated. A study of the optical system of this fish should be of value in this connection.

TABLE IV

Behavior of individual test males under the trials of Table I, expressed in percentage of positive reactions.

37	Test Male 1	2	ಣ	4	10	9	2	00	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Te 25 8	Tests Re-
\$\frac{37}{37}\$\frac{50}{50}\$\frac{100}{37}\$\frac{100}{50}\$\frac{100}{100}\$\frac{100}{	-	37			50	0	0	0		0	100	0	0	0	0	0	0	:	:	:	0		0	0	0	18-
37		50			100	100	0	0		0	100	100	100	0	0	100	001	:			0		0	33	0	53
37 50 0 -B 0 100 0 0 100 0		37			20	0	0	0		0	0	0		1 001	100	0	0				0	:	0	0	0	24-
87	4	3.7			50	0	0-B	0		0	100	0	0	0	0	100	0		:		0		0	0	100	+67
62 0 0 0 0 0 0 100 100 0 0 0 0 0 0 0 0 0	· Ma	200					0			0	100	100	100	0	0		100	:			0	:	0	33	0	90
33 0 0 50 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		62	: :		0	0	0	0		0	100	100	0	0	0	0	0	:			0	:	0	0	0	18
100 0 0 100 0 0 0 0 0 0 0 0 100 0 0 0 0	7	60	0	0	20	0	0	0	0	-	0		0	0		0	0	0	0	0	0	0	0	:	0	10
100 0 0 0 0 0 0 0 B 0 0 100 0 0 0 0 0 0	oc	100	0	0	100	0	0	0	0		100		0	100			100	0	0	0	0	0	0	:	0	30
100 100 0 0 0-B 0 100 100 100 0 0 0 0 0 0 0 0 0	6	100	0	0	0		0-B		0		100		0	100			100	0	0	0	0	0	0	:	100	33+
100 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		100	100	0			0		0		100		0	0			100	0	0	0	0	0	:	:	0	$^{31}+$
66 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0		100	0	0		:	0-B		0		100	:	100	100			100	0	0	0	0	0	:	:	0	37+
100 100 100 100 100 000 000 000 000 000		99	0	0	:	:	0	:	0		0		0	0			100	0	0	0	0	0	:	:	0	19-
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	100		17-17		100	14+		0	0	0	22	20	25	+88	I .		189		0	0	0	0	0	33+	- 17-	
	f Males							Ħ	,B	tten,	reg	arded	88	food	objec	t by t	est m	ale.								

of Males Reacting

Since Lebistes is an aggregating species living n communities of sometimes considerable size, a consideration of this habit is necessary in order to understand certain features of recognition. In the experimental portion of this paper, display and courting behavior were considered a positive evidence of sex recognition. Since the males will "court" a diverse number of objects, the question may be raised as to what is sex recognition in such forms, in the first place. It has been shown that males of other fishes, when in the proper physiological state, have a courting display for females indistinguishable from the fighting or "bluff" display. For example, Lissmann (1932) for Betta, Breder (1934) for Aequidens, and Noble (1934) for Eupomotis. Since Lebistes is continually ready for mating, there is every reason why these fishes should perform as they do on every occasion presenting itself. Whether these are to be considered bluff at one time and courtship another, seems to us to be almost pointless. Since these fishes do not fight as do the ones mentioned above, the display simply results in a parting of the two males. If both display, it would seem there is a mutually discouraging effect. If the approached fish is a female, it seems to make little difference, for she will flee also. Successful mating seems only to be accomplished by slipping up to the female, as previously pointed out, which interpretation leaves the display without functional significance. Since it might be argued that the display may have value in telling two approaching males what not to mate with, it is pointed out that males will sometimes pursue other males that in turn are bent on courting females. the latter male has its attention occupied, the former will sometimes apparently effect transfer. This naturally results in a complete but momentary interruption of the latter's courting Before these features become evident themselves, however, the simple, non-sexual, aggregating tendencies of Lebistes come into play.

The schools of *Lebistes* are certainly held together by the common means described by Parr (1927 and 1931), Spooner (1931), Bowen (1931) and Breder and Nigrelli (1935) for other fishes. In *Lebistes* the fishes do not head all the same way, as they are neither stemming a current (normally) nor moving in any more or less rectilinear path. Any such tendency is

broken up both by their individual browsing habits and the random sexual efforts of the males. Just as it has been shown that *Lebistes* will attempt to mate with a variety of objects, just so it may be shown that they will attempt to consort with practically any small moving object. In fact, the latter must take place first in order for the former to become operative.

The conclusion cannot be avoided that sex recognition, as such, is non-existent in Lebistes. Breder (1934) showed that in Aequidens recognition on a basis of behavior existed between ripe females and all other individuals. Because females of Lebistes are always capable of being "fertilized," even this distinction disappears. The sperm of this fish is encapsuled, and may be retained for months in a viable state in the female's body, and as the eggs are fertilized for at least as many as six successive broods, it would seem that here a distinct conservation of the male element occurs. This feature is completely nullified by the prodigious energy with which the males dispense their substance. Consequently it would seem that such fish are no more conservative in this regard than fishes that have a less efficient method of uniting sperm and eggs, but in which mating is only possible with physiologically suitable mates. It would seem that some level of effectiveness is reached in fish fertilization, but no matter by what means there always remains a loosely integrated element that makes for a large wastage of sperm. Perhaps this has some general but obscure physiological implication.

With the conditions as described a significance can scarcely be referred to the elaborate but variable pattern of the males. Certainly no female *Lebistes* gives the slightest evidence of ever being in a position to exercise any "sexual selection." As it might be thought that the lack of fixed pattern in the males of this species might be conditioned by this very fact, it may be pointed out that among the Poeciliidae there is a wide range of secondary differences between the sexes as well as many cases of nearly complete similarity. *Gambusia affinis*, for example, shows very little color or pattern differences between the sexes. Others show marked differences, but the male pattern is relatively definite and fixed; for example, *Micropoecilia branneri*. In some species the males carry elaborate ornamenta-

tion other than color, such as Xiphophorus and Mollienisia. In most, the males are considerably smaller than the females, but in some the corporeal differences are relatively slight, as in Platypoecilus. Since there is no reason to suppose that there is any important difference in the courtship and recognition mechanism of the various Poeciliidae, and a considerable amount of observation by both authors shows that the basic performances are similar, we have no reason to assume that their particular habits of courting tend to encourage (1) polymorphism of the male secondary sex characters; (2) fixity of the male secondary sex characters; (3) large differentiation between male and female, or (4) similarity between male and female.

Noble (1934) in discussing the possibility of sexual selection in Eupomotis, suggests that brighter males might be visited more frequently than relatively dull ones, or that the females might visit the more actively cleaned and presumably more conspicuous nests. He writes, "Hence it is probable that a true sexual selection may occur in the sunfish, since the females would presumably move into redds which attract their attention first." While this is not the place to discuss this view in detail, it may be pointed out that such a condition would appear to be valid only in the case of a large disparity between the number of males and females. Thus, a relatively few females, if mating with the first available males (on the average, most conspicuous), might become exhausted of roe before all nests received a quota of eggs. Observation by one of us in a scattered variety of places, over a number of years, leads to no such conclusion, however, since what may be called "bachelor" males have never been noted and the proportion of the sexes is certainly not low on the female side.

This matter is mentioned in the present connection to point out that for alleged sexual selection to be operative in fishes, even in forms that require cooperation of the female, there must be a sufficiently small number of females present to allow of the most "unattractive" males going unmated. Consequently in *Lebistes* and the Poeciliidae in general, even if it were not for the disinterest of the females, sexual selection could hardly be expected to be operative by that sex because of the complexion of the population which is so predominately female.

Pertinent to this discussion is the condition found in a large collection of living Lebistes sent to the New York Aquarium by Mr. Claudio Urrutia from Venezuela. The males of this strain were found to be strikingly lacking in the usually brilliant pattern of these fish. Some had the faintest suggestion of a yellow or greenish streak, some a dusky spot, but many were virtually of the same drab body tint as the female. These males were found to be, on a four month's observation, as sexually active as those of more brilliant strains and included the frequent completion of the sex act. It was noted, however, that offspring even from isolated females were few and irregular, although the activity of the adults was normal. This condition suggests the view that associates male secondary characters with the appropriate hormones and the corresponding physiological reproductive level, without reference to any possible selective value of ornamentation.

What is probably the most curious feature of the entire study is by what means the male locates the genital pore. In no case was there any hesitancy or any evidence of the employment of a trial and error method. How this is effected is not clear, and this study gives no clue, but it is to be noted that even in the attempted copulation with a shadow, exactly the appropriate region was selected.

Fertilization of an adequate nature by the exceedingly active males of this species is insured (1) by their aggregating behavior, which tends to hold them in a group; (2) by their sexual aggressiveness; (3) by finding females more frequently than males, because of their larger size and consequent greater visibility, and (4) by the countering actions of approached males.

SUMMARY

- 1. Sex recognition in *Lebistes reticulatus* Peters is feeble, if present at all, and sexually active males will attempt to fertilize a variety of objects.
- 2. Males isolated for one week reacted positively to: females exposed in the same aquarium; in an adjacent aquarium not more than 15 cm. distant; anesthetized females; males;

specimens of *Cyprinodon variegatus*, *Fundulus heteroclitus* and *Barbus conchonius*; and the projected shadows of living fish on the side of the aquarium, but not to carefully made models of females, either moving or still, to a mirror outside the aquarium (apparent distance too great?), or to females behind opaque but perforated screens.

- 3. Evidently vision alone accounts for the observed behavior.
- 4. It is inferred that discriminative sex recognition does not exist as such, but any object of appropriate size will stimulate the mating instinct if showing the characteristic motions of a living fish.
- 5. Female *Lebistes* have not been noted to display any interest in sex activity, as is common in forms that require cooperation of the sexes to insure reproduction.
- 6. In no case was there any error noted in locating the genital region by a courting male, the gonopodium always being thrust toward the region of the genital pore, including that of the projected shadow. The problem, in this species at least, then shifts from mate recognition to recognition of the genital region. The mechanism of the latter is not evident from these studies.
- 7. The sexes are primarily brought together by their non-sexual aggregating habits.
- 8. Adequate fertilization is insured by the great activity of the males and their general disposition to attempt mating with many objects showing slight motion. This is enhanced by both the preponderance of females and their ability to give birth up to six broods on one fertilization.
- 9. Detection of mating objects is entirely visual; chemical (taste and smell) and tactile (auditory and mechanical) senses do not enter at all.
- 10. No significance can be attached to the elaborate but variable colors of the males by this study.

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